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# ECOPHYSIOLOGICAL CHARACTERIZATION OF CULTIVABLE ANTARCTIC PSYCHROTOLERANT MARINE BACTERIA ABLE TO DEGRADE HYDROCARBONS

A. LO GIUDICE,* L. MICHAUD, G. GENTILE, M. DE DOMENICO, and V. BRUNI

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ABSTRACT. The basic understanding of both the physiology and ecology of psychrotolerant Antarctic bacteria is a crucial step for the optimization of their biodegradative activity in cold environments. The detection of cold-adapted hydrocarbon-degrading bacteria in Antarctic seawaters is certainly of great interest for bioremediative purpose in oil polluted marine Antarctic systems, where the introduction of non native species is not allowed. This study focused on psychrotolerant marine bacteria inhabiting an Antarctic coastal area directly influenced by the human activity at the Italian Research Station (Terra Nova Bay). Fifty bacterial strains were isolated from hydrocarbon-degrading enrichment cultures obtained from seawater samples collected in the inlet Road Bay (Ross Sea). A preliminary Restriction Fragment Length Polymorphism (RFLP) analysis, carried out on 16S rDNA amplified via PCR using RSAI and AluI restriction enzymes, was applied to cluster the isolates according to the restriction profile they showed. One representative isolate per cluster was selected for further characterization; to elucidate their taxonomic position, conventional phenotypic and phylogenetic analyses were performed. Results led to the identification of the isolates as members of ten genera belonging to four phylogenetic groups: the alfa- and gamma-proteobacteria subdivisions, the gram-positive branch and the *Cytophaga-Flexibacter-Bacteroides* (CFB) phylum. Results indicate a high degree of biodiversity within the peculiar ecophysiological group of the hydrocarbon-degrading marine bacteria.

## 1. Introduction

Hydrocarbons are potentially the most likely source of pollution in Antarctica where the anthropogenic impact is increasing more and more due to scientific and logistic activities. A number of studies have been focused on the chronic hydrocarbon contamination near the Antarctic research stations, revealing the presence and persistence of these human-derived contaminants [1, 2, 3, 4, 5, 6]. The need for research into hydrocarbon degradation process at low temperatures is therefore without question in order to preserve the vulnerable and extreme Antarctic environments. During the last two decades, many investigations have been carried out to establish the possible role of autochthonous microorganisms in the degradation rate of hydrocarbons [7, 8, 9, 10, 11, 12]. A small fraction of native bacterial species is able to grow on hydrocarbons, depending on environmental conditions but mainly on their adaptation to previous exposure to these pollutants [11]. A large amount of current

researches regards the biochemistry and genetics involved in the microbial biodegradation activity which seems to be a promising tool for biologically restoring environments contaminated with hydrocarbons [13]. Marine hydrocarbon-degrading bacteria may play an important role in the reduction of oil pollution in marine areas and therefore in the mitigation of the environmental impact deriving from the utilization of oil as a source of energy and chemicals [14]. Low temperature is the most important limiting factor for bioremediation activity; nevertheless, degradation of petroleum hydrocarbons by cold-adapted microorganisms has been reported by many Authors [15, 16, 4, 17, 18, 19, 20, 21, 22]. Recently, bacteria capable of hydrocarbon biodegradation have been isolated from both soil and seawater samples from Antarctica and some of them are taxonomically and physiologically novel [23, 4, 10, 20, 24, 14, 25, 26, 27, 22]. In this paper, we describe the isolation and preliminary characterization of hydrocarbon-degrading cold-adapted bacterial strains obtained from superficial Antarctic seawaters by enrichment on crude oil. This study is part of a larger interdisciplinary attempt at the University of Messina to advance the fundamental knowledge on the role of marine bacteria for the biodegradation of hydrocarbons in Antarctic waters.

## 2. Materials and Methods

*Bacterial strain isolation*. Surface seawater samples were collected in the inlet Road Bay (Ross Sea; $74°41.753S - 164°407.188E$) during the Antarctic summer of $1999 - 2000$. Enrichment cultures were carried out by supplementing $20$ ml samples with crude oil (Arabian Light; $0.1\%$, $v/v$) and nutrients [($NH_4$)$H_2PO_4$, $0.2\%$ , $w/v$]. Cultures were maintained in the dark at $4°$ C until transported in the laboratory for further analyses. Replicates $10$-fold dilutions of the original enrichment were made in ONR7a basal medium [28] supplemented with sterile Arabian Light oil ($0.1\%$, $v/v$). The tubes were incubated in the dark at $4°$ C until turbidity changes due to bacterial growth ceased (approx. $8$ weeks). Extinction cultures were obtained as a $10^{-4}$ dilution of the original enrichment and aliquots of them ($100$ $\mu$l) were subsequently plated in duplicate on solid ONR7a basal medium supplemented with individual hydrocarbons (crude oil or tetradecane or napthalene, $1\%$ final concentration) as a single carbon source. Plates were incubated at $4°$ C for about one month. All colonies grown on the initial medium were picked up for subsequent isolation and, in parallel, checked for growth in unamended ONR7a. All isolates belong to the collection of the National Antarctic Museum (MNA)Felice Ippolito at the University of Messina. They are maintained on Marine Agar (MA, Difco) slopes at $4°$ C and routinely streaked on agar plates from tubes every six months to control purity and viability. Antarctic strains are also preserved by freezing cell suspensions at $80°$ C in Marine Broth (MB, Difco) to which $80\%$ ($v/v$) glycerol is added. All media used in this study were sterilized by autoclaving at $121°$ C for $15$' and plates were incubated at $15°$ C for $14$ days, unless otherwise indicated. The analyses were performed at least twice.

*16S rDNA gene sequence determination and analysis of phylogenetic relationships*. Total genomic DNA was extracted from a $5$ ml late-exponential phase cell culture using the CTAB miniprep protocol [29]. PCR amplification of the 16S rRNA genes was performed with an ABI $9600$ thermocycler (PE Applied Biosystems) using the forward primer 16F27 ($5'$-AGAGTTTGATCMTGGCTCAG-$3'$) and the reverse primer 16R1492

(5′-TACGGYTACCTTGTTACGACTT-3′). The amplified products were purified with QI-Aquick PCR purification columns (Qiagen) following the manufacturers protocol. $10\mu l$ aliquot of each template were used for overnight digestion with a combination of the two restriction endonucleases AluI and RsaI (New England Biolabs). The digested amplified products were then screened by RFLP fingerprinting patterns. For each cluster obtained, two templates were selected at random for sequencing, with an ABI $310$ DNA Sequencer (PE Applied Biosystems) using the ABI Prism BigDye $2$ Terminator Cycle Sequencing ready reaction Kit (PE). Analysis of the sequences obtained was performed using $SIMILARITY\_MATRIX$ version $1.1$, $SEQUENCE\_MATCH$ version $2.7$ and $SEQUENCE\_ALIGN$ version $1.7$ from the Ribosomal Database Project (RDP) and BLAST [30] as previously described [24, 25]. The NEIGHBOR program was used to construct phylogenetic trees from the evolutionary distance matrices by the Neighbor-joining method [31].

*Physiological and biochemical characterization*. Pigmentation was recorded from growth on MA at $4^\circ$ C. The Gram reaction and the presence of endospores were determined by staining as described by [32]. The motility of young cells was determined by microscopically examining cell wet mounts (MB cultures were used). Flagellar arrangement was determined as described by [33], by using the Bacto Flagella Stain (Difco). Catalase production was assayed by using $H_2O_2$ $0.3\%$ with a colony taken from MA plates. Oxidase activity was determined by the method of Kovacs [34]. The growth of isolated bacteria at different temperatures was tested in MB incubated at $4$, $10$, $15$, $20$, $25$ and $30^\circ$ C up to $21$ days. The pH range for growth was determined in MB with pH values of separate batches of medium adjusted to $4$, $5$, $6$, $7$, $8$ and $9$ by the addition of $0.01$ M, $0.1$ M and $1$ M HCl and NaOH solutions. Salt tolerance tests were performed on Nutrient Agar with NaCl concentrations ranging from $0$ to $17\%$ $(w/v)$. The oxidative or fermentative utilization of glucose was tested by using the method of [35]. Testing the oxidative or fermentative acid production from carbohydrates (glycerol, mannose, maltose, galactose, starch and fructose) used the OF Medium (MOF, Difco) added with $10\%$ $(w/v)$ filter-sterilized test carbon source solution. Aliquots $(4.0$ ml) were dispensed in $5.0$ ml vials. Turbid suspensions $(0.2$ ml) of each strains were inoculated into each tube in duplicate. Turbidity and pH change were scored $3$, $7$ and $14$ d post-inoculation. Media lacking a carbon source were prepared as a negative control to account for any background growth. Chitin hydrolysis was assayed on the medium proposed by Brisou et al. [36]. Agarolytic activity was tested on the medium proposed by Vera et al. [37] and plates were incubated at $20^\circ$ C for $14$ days. Lipolytic activity was determined by incorporating Tween $80$ ($1\%$, $v/v$) into Sierra's medium [38] and plates were examined for opacity after $21$ days of incubation at $4$, $20$ and $30^\circ$ C. Starch hydrolysis was tested in a medium containing (per liter of distillated water): tryptone $10$ g, yeast extract $10$ g, $KH_2PO_4$ $5$ g, soluble starch $3$ g and agar $15$ g. Hydrolysis activity was detected by flooding the plates with Lugol solution. Additional biochemical and enzymatic tests were performed using API tests (BioMerieux), including API 20E (identification system for *Enterobacteriaceae* and other Gram-negative rods) and API 20NE (identification system for gram-negative nonenterobacterial rods) galleries. Both the test systems were prepared according to the manufacturer's instructions except that the bacterial strains were suspended in $2$ ml of a $3\%$ NaCl solution in the case of API $20$E system and that the suspension medium was substituted with presterilized seawater

for the conventional tests included in the API 20NE system. Isolates were tested for the ability to grow on various solid media such as Trypticase Soy Agar (TSA; Oxoid), TSA + $2.5\%$ $(w/v)$ NaCl; MacConkey agar (Difco) and TCBS agar (Difco). Susceptibility to antibiotics was tested by using the method of Bauer et al. [39]. Antibiotic-impregnated discs (Oxoid) were laid on MA plates which had been surface inoculated with the test strains. The following antibiotics were tested: penicillin G ($10\mu g$), polimixine B ($30\mu g$), nalidixic acid ($30\mu g$), tobramycin ($10\mu g$), tetracycline ($30\mu g$), chloramphenicol ($30\mu g$), and vibriostatic agent O/129 ($10\mu g$). Any sign of growth inhibition was scored as sensitivity to that antimicrobial compound. Resistance to an antimicrobial drug was indicated if it did not show any inhibition zone. This meant that resistance was strictly defined and results are reported as susceptible or resistant.

## 3. Results

*Bacterial strain isolation, 16S rDNA gene sequence determination and analysis of phylogenetic relationships*. Fifty bacterial isolates were obtained after culturing the Road Bay sample enrichments on parallel media. The preliminary RFLP analysis of bacterial isolates, carried out on 16S rDNA molecules amplified via PCR, allowed the subdivision into 12 clusters. The 16S rDNA sequences of one representative strain from each cluster were obtained and grouped into four bacterial phylogenetic branches, the $\alpha$- and $\gamma$-subdivisions of Proteobacteria, the gram-positive branch, and the Cytophaga-Flexibacter-Bacteroides (CFB) phylum (Table I). Overall, the phylogenetic analysis revealed that the twelve RFLP groups were representative of ten bacterial genera. Psychrotolerant isolates, affiliated to $\alpha$- Proteobacteria, were related to the genus *Sphingomonas*. Within the $\gamma$-Proteobacteria subdivision, the isolates were found to be related to *Marinobacter*, *Pseudoalteromonas*, *Halomonas*, *Ferrimonas*, *Acinetobacter*, *Shewanella and Marinomonas* genera. Finally, the strains no 1 and 2 were placed within the genus *Cytophaga* (CFB phylum) and *Rhodococcus* (gram-positive branch, *Actinomycetales*), respectively.

*Physiological and biochemical characterization*. Phenotypic characters are reported in Table II. Bacterial strains were predominantly gram-negative rods. A single polar flagellum was noted in eight gram-negative bacterial strains, which appeared motile under microscopy. The presence of endospore was never observed. The hydrolysis of almost one of the macromolecules provided was observed in all isolates with the exception of the strains no 3 and 11 (*Marinobacter* sp. and *Acinetobacter* sp., respectively). Strain no 16 (*Pseudoalteromonas* sp.) was able to hydrolyze all the macromolecules used for degradation test. Strains no 3 and 7 (both *Marinobacter* sp.) were not able to grow on the medium used for testing agarolysis. Likewise, the medium used for testing starch hydrolysis did not allow the growth of the strains no 11 and 15 (*Acinetobacter* sp. and *Sphingomonas* sp., respectively). Thus, hydrolytic capability towards agar and starch was not studied for these isolates. Although the majority of the isolates were capable of growing on chitin overlay plates, only one of them (namely the strain no 16, *Pseudoalteromonas* sp.) induced degradation of this macromolecule. All strains were lipolytic by means by hydrolysis of tween 80. Carbon source assimilation by the isolates was tested by using the API 20NE strips. Strains preferentially utilized glucose and malate, followed by maltose, citrate, caprate and mannitol. Only two isolates were capable of growth on mannose (strains no 1 and 2, i.e.

TABLE 1. 16S rDNA gene sequence affiliation of individual isolates to their closest phylogenetic neighbors (AC: Accession Number)

| $Strain$ | $Affiliation$ | $NeighborAC$ | $Taxonomy$ |
|---|---|---|---|
| 1 | *Cytophaga* sp. MBIC04666 | AB073583 | *CFB* |
| 2 | *Rhodococcus fascians* | X79186 | *Actinomycetales* |
| 3 | *Marinobacter* sp. DS40MB | AF199440 | $\gamma$-*Proteobacteria* |
| 6 | *Halomonas* sp. MYS2-06 | AJ414126 | $\gamma$-*Proteobacteria* |
| 7 | *Marinobacter* sp. NCE 312 | AF295032 | $\gamma$-*Proteobacteria* |
| 9 | *Ferrimonas balearica* | X93021 | $\gamma$-*Proteobacteria* |
| 11 | *Acinetobacter calcoaceticus* | AF458218 | $\gamma$-*Proteobacteria* |
| 12 | *Shewanella livingstonensis* | AJ300834 | $\gamma$-*Proteobacteria* |
| 13 | *Marinomonas* sp. TG7-01 | AJ414130 | $\gamma$-*Proteobacteria* |
| 15 | *Sphingomonas* sp. SW54 | U84838 | $\alpha$-*Proteobacteria* |
| 16 | *Pseudoalteromonas* sp. TG15 | AJ318942 | $\gamma$-*Proteobacteria* |
| 18 | *Pseudoalteromonas* sp. 22b | AF443784 | $\gamma$-*Proteobacteria* |

*Cytophaga* sp. and *Rhodococcus* sp.) and adipate (strains no 11 and 15, i.e. *Acinetobacter* sp. and *Sphingomonas* sp., respectively). None of the strains grew on phenyl-acetate. Many strains were oxidase positive. Enzyme arrangements were defined by the combination of the results obtained from API 20E and API 20NE systems. None of the strains was ornithine decarboxylase positive. Only the strain no 11, identified as *Acinetobacter* sp., was lysine decarboxylase positive. Indole production from tryptophane was never observed. $H_2S$ formation was recorded for the strains no 12 and 13 (both *Pseudoalteromonas* sp.). A number of isolates demonstrated the ability to produce acid from different sugars, alcohols and other carbon sources in oxidation/fermentation tests, which were performed by using API 20E system or MOF medium. In the majority of cases a growth without acid production was recorded. None of the strains was able to oxidize or ferment inositol, rhamnose, melibiose, arabinose and sorbitol. Acid production from amygdaline was never observed. Only the strains no 12 and 13, belonging to *Shewanella* sp. and *Marinomonas* sp. respectively, were able to oxidize and ferment the mannitol. Glucose fermentation by using the Lemos medium [35] was observed for the same two isolates. Most of the marine strains investigated were psychrotolerant when grown into MB medium. All strains grew well at $4^\circ$ C. Strain no 13 (*Marinomonas* sp.) failed to grow at $30^\circ$ C. The pH range for growth was generally from 5 to 9. In many cases growth occurred in a wide range of NaCl concentration. The strains no 1 and 2 (*Cytophaga* sp. and *Rhodococcus* sp., respectively) grew also without NaCl in the medium. The majority of the strains were susceptible to almost four of the antibiotics tested. All the strains were resistant to the O/129 agent. The strain no 1 (*Cytophaga* sp.) was susceptible only to chloramphenicol.

## 4. Discussion

One of the main objectives for the development and the optimization of bioremediation strategies is understanding the potential of pollutant-degrading microorganisms in the

TABLE 2. Morphological, biochemical and physiological characteristics of the isolates (nd: not determined; ng: no growth occurred on test medium; w: weak reaction; r: rod-shaped cells; c: coccoid cells; O: oxidation; F: fermentation; +: oxidofermentation; : growth without colour change in the medium)

| *Characteristics* | 1 | 2 | 3 | 6 | 7 | 9 | 11 | 12 | 13 | 15 | 16 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Gram reaction* | – | + | – | – | – | – | – | – | – | – | – | – |
| *Motility* | – | – | – | – | + | – | + | + | – | + | + | + |
| *Cell morphology* | r | r | c | c | r | r | r | r | r | r | r | r |
| *Polar flagellum* | – | – | – | – | + | – | + | + | – | + | + | + |
| *Colony pigmentation* | + | + | – | – | – | – | – | – | – | – | – | – |
| *Ensospores* | – | – | – | – | – | – | – | – | – | – | – | – |
| *Temp. range for growth 4-30 C* | + | + | + | + | + | + | + | + | – | + | + | + |
| *pH range for growth* | 5-9 | 5-9 | 6-9 | 5-9 | 6-9 | 5-9 | 5-9 | 5-9 | 5-8 | 5-9 | 5-9 | 5-9 |
| *NaCl conc. for growth (%)* | | | | | | | | | | | | |
| minimum | 0 | 0 | 3 | 3 | 3 | 3 | 3 | 0.5 | 0.5 | 3 | 0.5 | 1 |
| maximum | 9 | 9 | 5 | 17 | 17 | 17 | 17 | 7 | 7 | 15 | 11 | 13 |
| *Growth in the absence of NaCl* | + | + | – | – | – | – | – | – | – | – | – | – |
| *Utilization as a carbon source:* | | | | | | | | | | | | |
| Glucose | + | + | – | + | + | + | + | + | + | + | + | + |
| Arabinose | – | – | – | – | + | – | – | – | – | – | + | + |
| Mannose | + | + | – | – | – | – | – | – | – | – | – | – |
| Mannitol | + | + | – | + | + | + | – | + | + | – | – | – |
| N-acetyl-glucosamine | + | + | – | + | – | + | – | – | – | – | + | + |
| Maltose | – | – | + | + | + | + | + | + | + | + | + | + |
| Gluconate | + | + | – | + | – | + | + | – | – | + | – | – |
| Caprate | – | – | – | + | – | + | – | + | + | – | + | + |
| Adipate | – | – | – | – | – | – | + | – | – | + | – | – |
| Malate | + | + | – | + | + | + | + | + | + | + | + | + |
| Citrate | + | + | – | + | – | + | + | – | – | + | + | + |
| Phenyl-acetate | – | – | – | – | – | – | – | – | – | – | – | – |
| *Growth on various solid media* | | | | | | | | | | | | |
| TSA | + | + | + | + | + | + | + | + | + | w | + | + |
| TSA + 3% NaCl | + | + | + | + | + | + | + | + | + | w | + | + |
| TCBS | – | – | – | – | – | – | – | – | – | – | – | – |
| Mac Conkey | + | + | + | + | – | – | + | + | + | w | – | – |
| *Acid produced from:* | | | | | | | | | | | | |
| Glucose | – | – | – | O | – | O | – | F | F | – | O | O |
| Mannitol | – | – | – | – | – | – | – | + | + | – | – | – |
| Arabinose, amygdalin, inositol, melibiose, rhamnose, sorbitol | – | – | – | – | – | – | – | – | – | – | – | – |
| Sucrose | – | – | – | – | – | – | – | + | + | – | + | + |
| Glycerol | – | O | O | + | – | – | + | – | O | – | – | – |
| Maltose | + | + | + | + | – | – | + | + | + | – | – | O |
| Galactose | + | + | O | O | – | – | O | – | + | – | – | – |
| Mannose | O | O | O | O | – | – | – | – | + | – | – | – |
| Starch | + | + | + | + | – | – | O | – | O | – | – | O |
| Fructose | + | + | + | + | – | – | + | – | + | – | – | O |
| *Susceptibility to:* | | | | | | | | | | | | |
| Penicilline G | – | + | + | – | + | – | – | – | + | + | – | – |
| Polimixine B | – | – | + | + | + | + | + | + | + | w | + | + |
| O/129 | – | – | – | – | – | – | – | – | – | – | – | – |
| Nalidixic acid | – | – | + | + | + | + | + | + | + | w | + | + |
| Tobramicine | – | – | + | + | + | + | + | + | + | w | + | + |
| Tetracicline | – | – | + | + | + | + | + | + | + | w | – | + |
| Chloramphenicol | + | + | + | – | + | – | + | + | + | + | + | + |

TABLE 2 -Continued

| *Characteristics* | 1 | 2 | 3 | 6 | 7 | 9 | 11 | 12 | 13 | 15 | 16 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Biochemical tests:* | | | | | | | | | | | | |
| Urease | + | + | – | + | – | + | – | + | + | – | – | – |
| Lysine decarboxylase | – | – | – | – | – | – | + | – | – | – | – | – |
| Ornithine decarboxylase | – | – | – | – | – | – | – | – | – | – | – | – |
| $\beta$-galactosidase | – | – | – | + | – | + | – | + | + | + | + | – |
| Arginine dihydrolase | – | – | – | + | – | + | + | + | + | + | + | – |
| Oxidase | – | – | + | + | + | – | + | + | + | + | – | + |
| Catalase | – | – | – | + | – | + | + | – | – | + | – | – |
| Indole fomation | – | – | – | – | – | – | – | – | – | – | – | – |
| Voges-Proskauer reaction | + | + | – | – | – | – | – | + | + | + | + | – |
| Nitrate reduction | – | – | – | – | – | – | + | + | + | + | + | – |
| $H_2S$ formation | – | – | – | – | – | – | – | + | + | – | – | – |
| *Macromolecule hydrolysis:* | | | | | | | | | | | | |
| Aesculin | – | – | – | – | – | – | – | + | + | – | + | + |
| Gelatin | – | – | – | – | – | – | – | + | + | + | + | + |
| Chitin | – | – | – | – | – | – | – | – | – | – | + | – |
| Agar | + | + | ng | + | ng | + | nd | + | + | nd | + | – |
| Starch | – | – | – | – | + | – | ng | – | – | ng | + | – |
| *Tween 80 hydrolysis at:* | | | | | | | | | | | | |
| 4C | + | + | – | + | + | + | + | + | + | + | + | + |
| 15C | + | + | + | + | + | + | + | + | + | + | + | + |
| 30C | + | + | + | – | + | + | + | + | – | + | + | + |

environment by assessing their physiology and functions [40]. The detection and characterization of cold-adapted hydrocarbon-degrading bacteria in Antarctic seawaters is certainly of great interest for bioremediative purposes in oil polluted marine Antarctic systems, where the introduction of non native species is not allowed. Isolation of hydrocarbon-degrading bacteria from Antarctic soils has often been reported [41, 10, 42, 20, 11, 12, 43], while studies regarding their occurrence and ecology in Antarctic seawater are quite scarce [44, 25, 24, 27, 22]. Thus, our results confirm the presence of psychrotolerant hydrocarbon-oxidizing bacteria in Antarctic superficial seawater and enlarge the knowledge about their ecophysiology. Other studies have found that psychrotolerant bacteria vastly predominate in Southern Ocean seawater samples, with psychrophilic bacteria being rarely isolated [45, 46]. Microbial degradation of petroleum hydrocarbons is limited by low temperatures, but indigenous bacteria could be able to start promptly the biodegradative process in Antarctic seawaters. Psychrotolerant microbes, which are adapted to a wider temperature range, may have important advantages in biotechnological applications [47, 48] such as the removal of pollutants from cold environments. DNA-restriction analyses are frequently used for the rapid identification of bacterial species [49, 50, 51]. Results from RFLP analysis of PCR-amplified 16S rDNA led us to subdivide our isolates in twelve clusters, indicating a high biodiversity degree within the peculiar ecophysiological group of the hydrocarbon-degrading bacteria. These data are in agreement with a previous investigation regarding the study of both the inter- and the intra-specific biodiversity of 146

cultivable marine Antarctic bacteria which were clustered into $52$ groups [52]. Bacterial strains from the Road Bay superficial seawater were found to belong to four phylogenetic groups: the $\alpha$- and $\gamma$-Proteobacteria subdivisions, the gram-positive branch and the CFB phylum. The majority of the isolates (nine out of twelve) were affiliated to the $\gamma$-Proteobacteria group, highlighting the hydrocarbon-degradation potential of these bacteria as previously reported by many Authors for marine and soil environments [41, 13, 53, 26]. In particular, members of the genus *Acinetobacter* are known to produce biosurfactants that enhance the biodegradation rate on hydrocarbons by increasing the bioavailability of these hydrophobic water-insoluble substrates [54, 55, 56]. The only representative of the $\alpha$ -Proteobacteria group was identified as *Sphingomonas* sp. Bacterial strains of the genus *Sphingomonas* are well-known degraders of polycyclic aromatic hydrocarbons (PAHs) as sole source of carbon and energy; they are generally isolated from soils [57, 58, 59, 60], but also from seawaters [61, 62, 26] and marine sediments [63]. Hydrocarbon degradation by *Sphingomonas* has been reported also for low temperatures [42, 64]. In particular, a PAH-degrading *Sphingomonas* was isolated by Baranieki et al. [42] from fuel-contaminated Antarctic soil and was able to utilize the aromatic fraction of different crude oils, jet fuel and diesel fuel at low temperatures. Psychrotolerant isolates affiliated to the gram-positive branch were related to the genus *Rhodococcus*. Rhodococci are among the most promising hydrocarbon-degrading bacteria and, thanks to their enormous catabolic versatility, seem to be an interesting target for developing bioremediation techniques in cold environments [12]. Non-sporulating actinomycetes possess the capability to degrade a broad range of environmental pollutants, including a variety of aromatics, aliphatics and other xenobiotics [65, 66, 18, 67]. Several investigations have been performed on their alkane-catabolic pathways [18, 68, 69, 43], biosurfactants production [70, 26] and cell surface hydrophobicity [19]. Psychrotolerant alkane-degrading *Rhodococcus* spp. have been detected in Antarctic soils [10, 11, 12]. Nevertheless, as part of a survey of Antarctic bacteria able to degrade hydrocarbons, we have previously isolated marine variants of *Rhodococcus luteus* [22]. Psychrotolerant marine bacteria, because of their intrinsic advantage in the degradation of contaminants at low temperature and their ability to survive and continue their activity under *in situ* conditions, should be more efficiently exploited for the bioremediation of both temperate and cold environments than mesophiles or psychrophiles. The isolates characterized through the present study could be useful alternatives to chemical approaches in restoring Antarctic sites, where there exists a ban on the introduction of allochthonous species.

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Angelina Lo Giudice, Luigi Michaud, Gabriella Gentile, Maria De Domenico, Vivia Bruni
Dipartimento di Biologia Animale ed Ecologia Marina,
Università degli Studi di Messina
Salita Sperone, 31 V 98166 Messina (Italy)
* **E-mail**: alogiudice@unime.it

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